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Tissue repair
The hidden drama

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Key words: tissue repair, regeneration, scarring, wound healing, growth factors, extracellular matrix, stem cells

Abbreviations: cAMP, 3', 5' cyclic adenosine monophosphate; CDK, cyclin-dependent protein; DNA, deoxyribonucleic acid; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; GAG, glycosaminoglycan; KGF, keratinocyte growth factor; MAD, mothers against decapentaplegic; MMP, matrix metalloproteinase; IL, interleukin; JAK, janus kinase; OPN, osteopontin; PDGF, platelet-derived growth factor; RB, retinoblastoma; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor

As living beings that encounter every kind of traumatic event from paper cut to myocardial infarction, we must possess ways to heal damaged tissues. While some animals are able to regrow complete body parts following injury (such as the earthworm who grows a new head following bisection), humans are sadly incapable of such feats. Our means of recovery following tissue damage consists largely of repair rather than pure regeneration. Thousands of times in our lives, a meticulously scripted but unseen wound healing drama plays, with cells serving as actors, extracellular matrix as the setting and growth factors as the means of communication. This article briefly reviews the cells involved in tissue repair, their signaling and proliferation mechanisms and the function of the extracellular matrix, then presents the actors and script for the three acts of the tissue repair drama.

Proem

All the world's a stage,
And all the men and women merely players;
They have their exits and their entrances
And one man in his time plays many parts,
His acts being seven ages

Shakespeare
As You Like It
Act 2, scene 7, 139–143

The term "repair," when used in the context of the healing of damaged tissue, is defined as the restoration of tissue architecture and function after an injury. It encompasses two separate processes: regeneration and replacement. Regeneration refers to a type of healing in which new growth completely restores portions of damaged tissue to their normal state. Replacement refers to a type of healing in which severely damaged or non-regenerable tissues are repaired by the laying down of connective tissue, a process commonly referred to as scarring. While a few types of tissue injury (such as minor paper cuts) can sometimes be healed in such a way that no permanent damage remains, most of our tissue repair consists of both regeneration and replacement. Tissue repair may restore some of the original structures of the damaged tissue (such as epithelial layers), but may also result in structural abnormalities that impair organ function (such as the scar formed in the healing of a myocardial infarction).

Whether the healing of a wound proceeds down the regeneration or the replacement pathway (or both) depends, in part, on the type of tissue in which it occurs. Certain tissues of the body are more capable of cellular proliferation (and hence regeneration) than others. In this regard, there are three types of tissues: continuously dividing tissues, quiescent tissues and nondividing tissues. Continuously dividing tissues (also known as labile tissues) are comprised of cells that are constantly proliferating in order to replace dead or sloughed-off cells. Examples of such tissues include epithelia (such as skin, gastrointestinal epithelium and salivary gland tissue) and hematopoietic tissues. These tissues contain pools of stem cells, which have enormous proliferative and self-renewing ability, and which give rise to more than one type of cell. Replicating asymmetrically, each stem cell gives rise to one daughter cell that differentiates and matures and another daughter cell that remains undifferentiated and capable of beginning another self-renewing cycle.

Some tissues, known as quiescent tissues (or stable tissues) are composed of cells that normally exist in a non-dividing state but may enter the cell cycle in response to certain stimuli, such as cell injury. Tissues falling into this category include parenchymal cells of the liver, kidney and pancreas, mesenchymal cells such as fibroblasts and smooth muscle cells, endothelial cells and lymphocytes. It should be noted that the liver, unlike other quiescent tissues, has a relatively robust proliferative capacity. When a lobe

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of the liver is resected for donation, for example, the remaining liver cells proliferate at such a rate that the liver reaches a size similar to that prior to resection. While this process is commonly described as regeneration, it is more accurately viewed as compensatory growth, since the original lobe itself does not regrow. A few types of tissue are composed of cells that have left the cell cycle permanently, and are therefore unable to proliferate. These nondividing tissues (or permanent tissues) include cardiac and skeletal muscle. Tissue repair in these tissues always leaves permanent evidence of injury, such as a scar.[1]

Dramatis Personae

The cast of characters in the tissue repair drama is large and varied. Here we list the major actors, with a focus on those that may be less known to the reader. Central in the drama are the tissue's own lost or damaged cells, which in most cases are terminally differentiated and incapable of replication. In nondividing tissues, such as myocardial tissue, lost cells are simply never replaced. In other tissues, however, replacement is possible. Continuously dividing tissues are particularly adept at self-renewal, undergoing innumerable cycles of cell loss and replacement during a normal human lifespan. The regenerative capacity of these tissues lies not in their parenchymal cells (which are terminally differentiated and thus unable to replicate), but in stem cells located deep within the tissue.

Stem cells are unique for two reasons: (1) they have the ability to self-renew and (2) they have the capacity to generate more than one cell type. Self-renewal occurs either by symmetric replication (in which a stem cell gives rise to two daughter stem cells, equally capable of self-renewal) or asymmetric replication (in which one daughter cell remains a self-renewing stem cell and the other daughter cell differentiates and matures).

The capacity of a stem cell to give rise to multiple lineages of cells is most striking in embryonic stem cells. These cells, which are denoted as pluripotent, are capable of generating cells from any of the tissues of the body. Adult (or somatic) stem cells are designated as multipotent, and give rise to a more restricted array of cell types. As expected, somatic stem cells have been found in continuously dividing tissues, such as skin, gastrointestinal epithelial lining, cornea and hematopoietic tissue. However, they have also been discovered in certain quiescent tissues, such as liver, pancreas and adipose tissue, in which they do not normally produce differentiated cells. Most surprising is the recent discovery of stem cells residing in certain parts of the central nervous system, an organ system whose tissues have long been thought to be incapable of proliferating.[2]

Most somatic stem cells are located in niches, micro-environments within a tissue comprised of both stem cells and non-stem cells. Neighboring non-stem cells signal the stem cell to divide when necessary, a task that the stem cell generally performs very slowly. In the skin, stem cells located in a niche within the hair follicle bulge give rise to all the cells comprising the hair follicle and contribute to the production of new surface epithelial cells after wounding.[3] Stem cells of the small intestine are located within monoclonal, stem cell-derived crypts that are completely regenerated every 3 to 5 days. The bone marrow contains not only hematopoietic stem cells, which give rise to all blood cell lineages, but also multipotent stromal stem cells, which travel to different regions in the body and generate chondrocytes, osteoblasts, adipocytes, myoblasts and endothelial cells. These stromal stem cells participate in cell replenishment after tissue injury, but they do not seem to have a function in normal tissue homeostasis.

Beyond the stem cell, three other types of cells are critical to the process of tissue repair: fibroblasts, endothelial cells and macrophages. In most wounds, complete replacement of wounded tissue to its original, unharmed state is impossible. The wound must therefore be healed using externally obtained material to reconnect the viable tissue margins. This process, discussed in detail later, involves the laying down of acellular fibrous tissue to replace the region of lost cells. The fibrous tissue is laid down by fibroblasts, which migrate to the injured area, proliferate and secrete collagen under the influence of numerous growth factors and cytokines. In the earliest stages of wound healing, fibroblasts are few and far between, suspended together with tenuous new blood vessels in an edematous pink substance termed granulation tissue. Initially the new blood vessels are critical in the transport of nutrients and cells to the new tissue, but after a time, they recede along with the fibroblasts, leaving a collagenous scar that is remodeled and strengthened over time. Macrophages are essential directors of this drama, secreting growth factors that entice and stimulate fibroblasts, endothelial precursor cells and (in skin wounds) keratinocytes. They also oversee the deposition and remodeling of extracellular matrix material.

The Script

The script of the tissue factor drama—the migration and proliferation of cells, the laying down of extracellular matrix, and the remodeling of collagen to form a durable scar—is carried out by a process known as receptor-mediated signal transduction. Akin to the words spoken between people, ligands such as growth factors and cytokines float between cells, carrying directives to perform a certain action. Cells "hear" these words when the ligands bind to cell-surface receptors, which bring the message into the cell, resulting in a new action, such as migration, proliferation or secretion of a substance. Herein we will discuss the words spoken between the actors, the way the actors hear these words and the manner in which the message gets to the cell nucleus in order to effect change.

Growth factors and cytokines. Growth factors are specialized polypeptide molecules that bind to receptors on target cells and deliver messages regarding migration, proliferation, differentiation, survival and secretion. The list of growth factors and their attendant functions is so long that it would tax even the most capable memorizer. Herein we limit our discussion to the primary growth factors associated with each stage of tissue repair (**Table 1**).

One of the most critical growth factors in tissue repair is transforming growth factor beta (TGF-β). This growth factor belongs to a superfamily that also includes a number of other factors with wide-ranging functions, such as bone morphogenetic proteins,

Table 1. Major steps in wound healing and their associated growth factors

	EGF	FGF	KGF	PDGF	TGF-α	TGF-β	TNF	VEGF
Fibroblast migration		X		X		X		
Fibroblast proliferation	X	X		X	X		X	
Monocyte migration		X		X		X	X	
Macrophage activation							X	
Epithelial migration	X	X	X		X			
Epithelial proliferation	X	X	X		X			
Angiogenesis		X		X	X		X	X
Collagen synthesis				X		X		
Collagenase synthesis	X	X		X			X	X
Wound contraction		X		X				

EGF, epidermal growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

activins, inhibins and Müllerian inhibiting substance.[4] Made by platelets, endothelial cells, lymphocytes and macrophages, TGF-β binds to cell-surface receptors that have serine/threonine kinase activity, triggering phosphorylation of cytoplasmic transcription factors called Smads, so-named for the two drosophilia proteins of which they are homologs: the mothers against decapentaplegic (MAD) and *Caenorhabeitis elegans* proteins.[5] Smads then enter the nucleus and affect gene transcription.

Although TGF-β has many functions, its most important role in tissue repair is to promote fibrosis, a feat it accomplishes by: (1) attracting fibroblasts and stimulating them to proliferate, (2) triggering fibroblasts to secrete collagen and (3) inhibiting extracellular matrix degradation by metalloproteinases. Not surprisingly, TGF-β is a key factor in conditions involving pathologic fibrosis, such as pulmonary fibrosis, systemic sclerosis and Marfan syndrome.[6] In addition to its fibrogenic effects, TGF-β also inhibits epithelial cell growth, diminishes inflammation and promotes invasion and metastasis in tumors.[7]

Another essential, multitasking growth factor is platelet-derived growth factor (PDGF), so named because it is stored in platelet granules and released upon platelet activation. Made by a number of cells including macrophages, endothelial cells and smooth muscle cells, PDGF is involved in nearly every aspect of tissue repair. It calls neutrophils, macrophages and fibroblasts to the wound area and subsequently stimulates and activates them. It also induces angiogenesis, triggers production of matrix metalloproteinases (MMPs), fibronectin and hyaluronic acid and aids in wound contraction.[8]

Fibroblast growth factor (FGF) calls virtually all of the major players (macrophages, fibroblasts and endothelial cells) to the scene of the wound. It initiates the migration of epithelial cells in from the margins of the wound, mediates wound contraction and stimulates angiogenesis.[9] Contrary to what one might infer from its name, however, FGF does not stimulate collagen synthesis. The more appropriately-named vascular endothelial growth factor (VEGF) acts primarily on endothelial cells. It is a potent inducer of blood vessel formation in tissue repair as well as in early fetal development.[10] Overexpressed in certain tumors, particularly renal cell carcinoma, VEGF is a target for the development of new chemotherapeutic agents.[11] Other growth factors include epidermal growth factor (EGF) and hepatocyte growth factor (HGF). Both stimulate epithelial cell and hepatocyte proliferation and enhance epithelial cell migration. Once known as the scatter factor, HGF also promotes cell scattering during embryonic development.[12]

In addition to growth factors, cytokines also carry important messages between cells during tissue repair. Small proteins secreted by cells of the immune system, cytokines are perhaps best known for their role as immunomodulators within both the cellular and humoral arms of the adaptive immune system. Three cytokines in particular are involved in tissue repair: tumor necrosis factor (TNF) and interleukin-1 (IL-1) participate in the wound healing stage of tissue repair and TNF and interleukin-6 (IL-6) are involved in liver regeneration.[13]

Receptor-mediated signaling. For humans, words must be seen or heard to have meaning. So it is with the messages between cells in the tissue repair drama: the growth factor or cytokine message must have a way to get into the target cell for action to take place. This process is termed receptor-mediated signal transduction and it involves binding of a ligand (a growth receptor or cytokine) to a receptor molecule on a target cell. This binding triggers events that transduce the signal from the outside of the cell to the inside. The end result is a change in gene expression, for example, transcription of genes that normally may be silent, such as those that control cell cycle entry.

Receptor-mediated signaling often occurs between cells adjacent to one another (paracrine signaling); for example, macrophages produce growth factors that bind to receptors on adjacent fibroblasts. However, signaling can also occur between cells located at some distance from each other (endocrine signaling), as is often the case with cytokines and their distant targets. Signaling may even occur within the same cell (autocrine signaling), as when a tumor cell overproduces both a growth factor and its receptor, which interact and lead to unbridled cell growth.

Whatever the mode of signaling may be, there are four main types of receptors: those with intrinsic tyrosine kinase activity, those without intrinsic tyrosine kinase activity, G protein-coupled receptors and steroid hormone receptors. Receptors with intrinsic tyrosine kinase activity are common targets for growth factors. When a growth factor binds to this type of receptor, the receptor dimerizes, causing phosphorylation of tyrosine residues and activation of the receptor. The activated receptor in turn phosphorylates other molecules, inducing them to carry the message (or "signal") into the nucleus, resulting in a change of gene expression. Examples of actions mediated by this type of receptor include: production of growth factors, production of growth factor receptors, production of proteins that control entry of the cell into the cell cycle and activation of cell proliferation and survival (through inhibition of apoptosis).[14]

Receptors lacking intrinsic tyrosine kinase activity use a similar phosphorylation-activation system to transmit messages into the cell; however, since they lack phosphorylating capability, they must recruit a separate kinase system, called the JAK-STAT pathway, to complete the job. The receptor transmits the signal from the ligand (often a cytokine) on the surface of the cell to a Janus kinase (JAK) protein inside the cell. Once activated, the JAK protein in turn phosphorylates and activates cytoplasmic transcription factors called STATs (signal transducer and activator of transcription). As their name implies, STATs transduce the signal from the JAK protein to the nucleus of the cell, where they activate the transcription of certain genes.[15]

The largest family of signal transduction cell membrane receptors is comprised of G protein-coupled receptors. Many different types of ligands bind to this type of receptor, such as chemokines, vasopressin, serotonin, histamine, epinephrine, norepinephrine, calcitonin, glucagon, parathyroid hormone, corticotropin and rhodopsin.[16] When the receptor receives a signal from a ligand, its seven transmembrane alpha helices change conformation, allowing it to interact with a G protein. Once activated, the G protein transmits the signal to a second messenger molecule, such as 3', 5' cyclic adenosine monophosphate (cAMP), which carries the signal to the nucleus of the cell. Such cAMP-mediated pathways are involved in vision and olfactory sensing. Steroid hormone receptors are unique in that they are located inside the cell rather than on the cell membrane. Their ligands, which include steroid hormones, thyroid hormone, vitamin D and retinoids, must diffuse through the cell membrane in order to bind to the receptor, which binds to DNA and alters gene expression.

The cell cycle. One of the main actions in the tissue repair script is cell proliferation. In order to heal after injury—whether by regeneration or scarring—cells must enter and progress through the cell cycle, a tightly-regulated process that consists of two main activities: DNA replication and mitosis. Continuously proliferating cells are always moving through the cell cycle, whereas quiescent cells must be called into the cell cycle by growth factors or cytokines (via receptor-mediated signal transduction) or by ECM components (via integrins).

The cycle consists of four consecutive phases: the G_1 (presynthetic) phase, the S (DNA synthesis) phase, the G_2 (premitotic) phase and the M (mitotic) phase. Cells may begin their journey through the cycle from G_1 or G_0 (a resting phase outside the cell cycle). In order to move from G_0 to G_1, cells must activate numerous previously-silent genes including proto-oncogenes, genes required for ribosome synthesis and genes required for protein translation. To move into the S phase and begin the process of replication, cells must pass a checkpoint at the end of G_1. Several such checkpoints built into the cycle operate as quality-control monitors, validating that the cell has accurately completed one phase of the cell cycle before allowing the cell to progress to the next phase. The G_1 checkpoint assesses the integrity of DNA before allowing cells to pass through to S phase. It also serves as a decision point, determining whether the cell should divide immediately, divide at a later point or enter a resting stage. Another checkpoint at the end of G_2 evaluates DNA after replication to see if the cell can safely enter mitosis. A separate metaphase checkpoint monitors the alignment of chromosomes at the mitotic plate and when alignment is achieved, allows chromosomes to separate into their sister chromatids. After splitting into two daughter cells, the cell again enters G_1.

Because the correct functioning of the cell cycle is crucial to its integrity, there are multiple controls built into the system, including activators, inhibitors and sensors that control checkpoints in the cycle. Proteins called cyclins, together with associated cyclin-dependent protein kinases (CDKs), drive the cell cycle by phosphorylating proteins that are essential for cell cycle transitions.[17] One such protein is the retinoblastoma (RB) protein, which in its normal, unphosphorylated state stalls the cell at the G_1/S transition by binding and inactivating the transcription factor E2F.[18] When RB is phosphorylated by cyclins, it releases (and activates) E2F, allowing transcription of genes necessary for cell cycle progression. Cyclin proteins are themselves regulated by CDK inhibitors.

The Stage

The stage upon which the drama unfolds is a special type of tissue known as extracellular matrix (ECM). ECM exists in two forms: interstitial matrix (a gelatinous, amorphous intercellular material) and basement membrane (a thin, highly-organized, plate-like layer upon which epithelial cells rest). Although it may appear inert and static, ECM has a long list of responsibilities and functions. Beyond its obvious physical characteristics—imparting resilience to soft tissues and firmness to bone—it also stores and presents growth factors, acts as a scaffold to which migrating cells can adhere and establish polarity and facilitates cell growth both in physiologic and tissue repair settings. ECM is the ever-changing background for regeneration and wound healing, but it also accompanies other processes such as morphogenesis, chronic fibrotic processes and tumor invasion/metastasis.

The many constituents of the ECM can be grouped into three types of molecules, according to their main functions: structural molecules (such as collagen and elastin), adhesive molecules (such as integrins, fibronectin and laminin) and resilient components (such as proteoglycans and hyaluronan). The interstitial matrix is composed primarily of collagen, elastin, fibronectin, proteoglycans and hyaluronan, whereas the basement membrane is composed largely of nonfibrillar collagen, laminin and proteoglycans.[1] A short description of each major ECM component follows.

Collagen, the most common protein in the animal world, consists of three chains that combine to form a triple helix trimer. Although 27 types of collagen have been identified, less than half of these have major roles in the human body. Fibrillar collagens (types I, II, III, V and IX) are found in many types of hard and soft tissues. Type IV collagen, which is arranged in long sheets rather than fibrils, is a primary component of basement membranes. Collagen imparts strength to tissues, but the ability to stretch and snap back into shape is provided by elastic fibers. Elastic fibers are composed of a central core of elastin surrounded by fibrillin, a glycoprotein that controls the availability of TGF-β within the ECM.

Adhesive molecules, such as integrins, fibronectin and laminin, provide connections between cells or between cells and ECM components. Integrins are transmembrane glycoproteins with an extracellular domain that attaches to ECM components, such as laminin and fibronectin and an intracellular domain that links to cytoskeletal complexes.[19] They are operative in the earliest stages of wound healing, helping leukocytes adhere to vascular endothelium in preparation for their transmigration through the vessel. Fibronectin helps stabilize the initial blood clot filling the wound by binding to fibrin; it also provides a framework for building the collagen matrix during wound healing. Laminin provides a connection between cells and the ECM; it also binds with type IV collagen to form the basement membrane.

The resilient nature of the ECM is provided by proteoglycans and hyaluronan. Both of these substances are composed of glycosaminoglycans (GAGs), long repeating polymers of disaccharides. Proteoglycans, which consist of GAGs linked to a protein core, have many different roles: regulation of ECM permeability, mediation of inflammatory and immune responses and control of cell growth. Hyaluronan, comprised of many repeats of a single dissacharide, binds huge amounts of water and provides strength and turgor to connective tissue.

The Drama

Preface: regeneration vs. scarring. True regeneration, in which new cells replace damaged or dead cells such that the tissue is restored to its original state, occurs infrequently in humans. Two conditions must be met for a tissue to undergo pure regeneration (without scarring): (1) the injured cells must be capable of proliferation (as is the case in continuously dividing and quiescent tissues) and (2) the underlying stromal framework must be intact.

Regeneration occurs in a physiologic manner in continuously dividing tissues. Sloughed-off gastrointestinal epithelial cells, for example, are replaced by new cells arising from stem cells in the intestinal crypts. Pathologic examples of pure regeneration, however, are few and far between. One example occurs when the liver undergoes acute, toxic injury from acetaminophen overdose. In this type of injury, hepatocytes are destroyed, but the underlying stromal framework remains intact, allowing for the possibility of complete regeneration of the injured tissue.[20]

In a process akin to regeneration, some organs are able to grow in size in response to resection. If a lobe of the liver is resected, the remaining liver will grow larger in response. A wave of replication occurs as hepatocytes are pulled from their quiescent state and enter the cell cycle. Following a second wave of replication in nonparenchymal cells (such as Kupffer cells and endothelial cells), hepatocytes return to their quiescent state. This process, though often termed "regeneration," is not true regeneration, but compensatory growth. True regeneration of lost organs is, at this time, a process relegated to certain animal species and science fiction movies, but perhaps in the not-so-distant future stem cells will be used for this purpose.[21]

If an injury damages only parenchymal cells in a continuously-dividing or quiescent tissue, repair by regeneration is possible. However, if an injury is so severe as to damage not only the parenchymal cells but also the underlying stromal framework of the tissue (as occurs in most injuries) or if the injury occurs in non-dividing tissues, regeneration is impossible. In these instances, the tissue is repaired by the deposition of fibrous tissue in the defect created by the wound. Regeneration involves restitution of tissue components; repair involves "patching" rather than restoring. The amount of regeneration vs. repair that occurs depends on the proliferative capacity of the cells, the integrity of the stromal framework and the duration of the injury and inflammatory response.[1]

Repair by connective tissue involves the influx of debris-removing inflammatory cells, formation of granulation tissue (a substance consisting of fibroblasts and delicate capillaries in a loose extracellular matrix) and conversion of said granulation tissue into fibrous tissue that is remodeled over time to form a scar. There are five major components in this process: inflammation, new vessel formation, fibroblast proliferation, collagen synthesis and scar remodeling.

Though the general principles are the same no matter what type of wound is being healed, the extent of granulation tissue, inflammation and scarring vary depending on the size and type of wound. In the case of skin wounds, there are two types of wound healing: first-intention healing and second-intention healing.[22] Wounds healing by first intention are relatively small, with limited epithelial and connective tissue damage, such as paper cuts, well-approximated surgical incisions and superficial stab wounds. Healing in this type of wound is generally rapid, since the area of the defect is relatively small. Inflammation and granulation tissue are present but not abundant and scarring is minimal.

In contrast, wounds healing by second intention are larger wounds with margins that are not easily approximated, such as burns, infarctions, ulcers and large excisional skin wounds. This type of wound necessarily heals more slowly, as the defect to be repaired is larger. The abundance of fibrin, necrotic debris and exudate necessitates a more intense inflammatory response and consequently the chance of inflammation-mediated injury is greater than it is in first-intention healing. Granulation tissue is abundant and wound contraction and scarring is maximal. Despite these differences between first and second intention healing, the underlying drama is very similar. We will present the

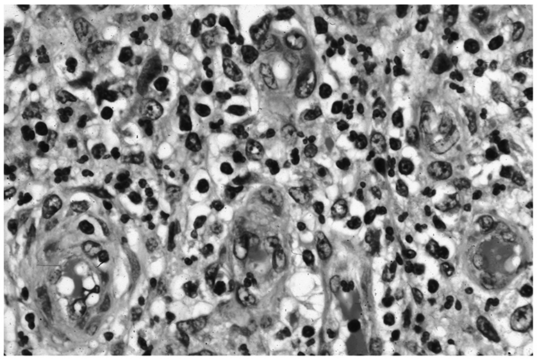

Figure 1. Skin ulcer showing early-stage granulation tissue with numerous blood vessels and scattered fibroblasts in a loose extracellular matrix. Numerous neutrophils are present within the granulation tissue.

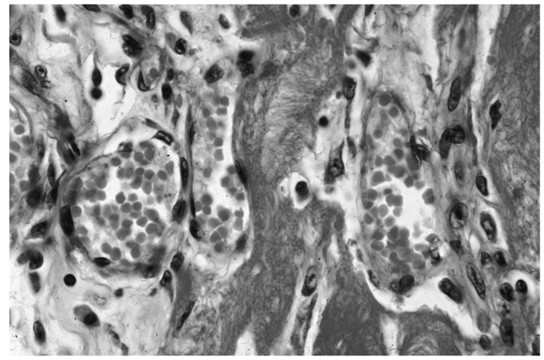

Figure 2. Early granulation tissue with blood vessels in a loose matrix of collagen and fibrin.

drama as it occurs in first intention wounds, noting second intention differences where necessary.

Act I: inflammation. Immediately after the wound is inflicted, the most urgent task is not to repair the damaged tissue, but to stop the flow of blood from the wound. Within seconds of the injury, exposed collagen activates coagulation in the region of the injury. Platelets form an initial plug, coagulation cascade factors interact to form fibrin (which seals and solidifies the plug) and a blood clot soon appears at the skin surface. Not only does the clot stop the bleeding, but it serves as a scaffold for incoming cells and a repository of cytokines and growth factors.[22,23]

At the skin surface, all appears static and calm as the clot dries and forms a scab. Underneath the surface, however, a flurry of activity begins. An army of neutrophils rushes to the scene through dilated local blood vessels, called to the area by IL-1, TNF, TGF-β and other growth factors. Within 24 hours, the neutrophils appear at the edges of the wound incision, using the fibrin scaffolding of the clot to invade the region of destruction, their primary tasks being to break down debris and kill bacteria.

Epithelial cells, meanwhile, begin their arduous process of repair by crawling from the basal layers of the epidermis into the wounded region, depositing basement membrane components as they migrate. They are called to begin this process by EGF and TGF-α, secreted by activated platelets and macrophages. In addition, fibroblasts secrete KGF (keratinocyte growth factor) and IL-6, factors that enhance keratinocyte migration and proliferation.[24,25] Fusing in the midline underneath the inert scab, these epithelial cell pioneers form the first thin, continuous layer of epithelium upon which the rest of the new epithelium will be built.[26]

Act II: proliferation. Following the initial flurry of clotting and neutrophil activity, the work of cell proliferation begins in earnest. The characteristic feature of this act in the drama is the formation of a substance called granulation tissue, a pink, soft material so-named for its irregular, grainy appearance. Consisting of new blood vessels and fibroblasts in an extracellular matrix, granulation tissue is laid down over a period of a few days, beginning with a few small, tenuous blood vessels and scattered fibroblasts. Initially, the new vessels are leaky and the granulation tissue has an edematous, loose appearance (**Fig. 1**). As the vessels grow stronger and fibroblasts begin to lay down collagen, it takes on a more substantial appearance, reaching its maximum vascularity by day 5.[1]

Macrophages—large, multitasking cells derived from monocytes—now replace the neutrophils that were so numerous in the immediate aftermath of the wounding. As their name suggests, a primary function of macrophages is the ingestion of unwanted materials: bacteria, cell remnants, debris, fibrin and foreign material. But macrophages also serve as directors for many other parts of the drama. They call in and stimulate fibroblasts and keratinocytes (through release of PDGF, TGF-β, TNF, IL-1 and KGF), stimulate angiogenesis (by secretion of VEGF, FGF and PDGF) and direct the laying down and remodeling of extracellular matrix [through the use of TGF-β, PDGF, tumor necrosis factor (TNF), osteopontin (OPN), IL-1, collagenase and matrix metalloproteinases (MMPs)].[22]

The formation of new blood vessels (angiogenesis) begins within the first few days following injury, aided by VEGF and FGF.[27] The process is unexpectedly aided by a small number of bone-marrow-derived endothelial precursor cells which home to the wound by mysterious mechanisms. Their contribution to the overall angiogenesis effort, however, is minimal.[28] Formation of the correct blood vessel structure is aided by integrins on the surface of endothelial cells; surrounding perithelial cells are recruited with the help of angiopoietin.

The end point toward which the entire drama is directed is the formation of a scar, a strong collagen filler that bridges the gap left by tissue destruction, restoring strength and integrity to the tissue. This process begins with the entrance of the fibroblast between days one and three, called to action by many growth factors and cytokines, including PDGF, EGF and TGF-β. At first, the type III collagen secreted by fibroblasts is arrayed vertically at the margins of the incision. Later, the collagen combines with fibrin and plasma fibronectin (which deposition is promoted by the vascular permeability inherent in angiogenesis); this provisional matrix provides a framework for further fibroblast and endothelial cell ingrowth (**Fig. 2**).[29]

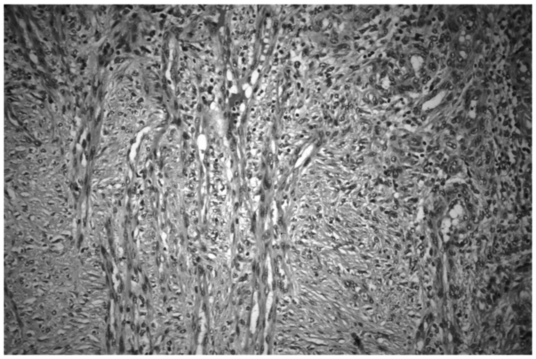

Figure 3. Skin ulcer showing later-stage granulation tissue with blood vessels and early collagen deposition.

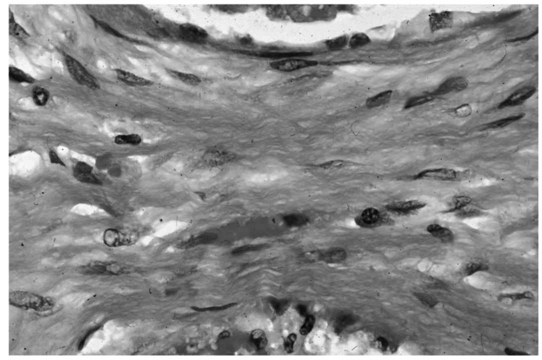

Figure 4. Early scar formation showing fibrillar collagen, plump fibroblasts and spindle-shaped fibrocytes.

By day 3 to day 5, ECM formation is well underway, with granulation tissue serving as a substrate (**Fig. 3**). Shards of elastic tissue form within the wound site. Plump fibroblasts mature into thin, spindle-shaped fibrocytes, which become entrapped in the dense, fibrillar collagen they have secreted (**Fig. 4**). Collagen deposition is a balance between synthesis and degradation. In these early stages, the balance tips toward collagen synthesis, aided by TGF-β, which inhibits the degradation of collagen. The process of ECM deposition ends within 2–3 weeks.

Meanwhile, the epithelium quietly continues to proliferate. Mediated by the aforementioned KGF and IL-6, it increases in thickness, eventually regaining its full, stratified architecture and keratinized surface some time between 1 and 3 weeks (or later in second-intention healing).[27]

Act III: maturation. During the second week following the incision, leukocytes gradually abandon the wound area and vessels recede (**Fig. 5**). Type I collagen synthesis increases, due to both an increased number of fibroblasts and an increased rate of synthesis per fibroblast.[30] Where there was once granulation tissue, there is now a preliminary scar, pale and firm, composed of dense collagen, fibroblasts and shards of elastic tissue. In another two weeks, even the fibroblasts begin to disappear and eventually an acellular scar bridges the region of the wound, covered by intact, fully-developed epidermis. Dermal appendages in the region of the wound, however, are not reformed.

Over the next several weeks, the composition of the extracellular matrix changes, remodeling the newly-formed scar such that it attains maximal strength at an appropriate size. Although several serine protease enzymes (such as neutrophil elastase, cathepsin G, kinins and plasmin) operate in the remodeling process, the key mediators of remodeling are zinc-dependent proteases known as matrix metalloproteinases (MMPs).[31] There are many different types of MMPs, including collagenases (which cleave fibrillar collagen), gelatinases (which act on amorphous collagen and fibronectin) and stromelysins (which degrade a number of ECM components including laminin, fibronectin, proteoglycans and amorphous collagen). MMPs are produced by many different types of cells, including fibroblasts and macrophages; their secretion is stimulated by PDGF and FGF and inhibited by TGF-β. Once they are produced, MMPs are rapidly broken down by a family of enzymes known as tissue inhibitors of metalloproteinases (TIMPs). Produced by mesenchymal cells, TIMPs help reign in collagen (and other ECM component) breakdown so that scar formation can proceed.

Ultimately, strength is achieved by increased collagen synthesis, followed by post-synthetic modifications of collagen, including cross-linking and increased fiber size. Early in wound healing, the collagen is relatively thin and oriented parallel to the skin; over time, the initial collagen fibrils are resorbed and replaced with thicker fibrils aligned with stress lines.[22] Wound strength increases rapidly during the first month, then slows, reaching a plateau of 70–80% of the original tensile strength by the end of the third month. Although full-thickness epithelial regeneration is possible, any skin appendages lost during the initial injury will not be reformed.

Given their easily-approximated margins, wounds healing by first intention generally have minimal scarring. Wounds healing by second intention, however, are larger, with more epithelial destruction, necessitating more collagen deposition to close the wound. The process of wound contraction eases the fibroblasts' burden a bit. Specialized contractile, smooth-muscle-like cells called myofibroblasts appear within the wound and pull the margins of the wound toward each other. These cells may be derived from tissue fibroblasts under the influence of FGF and PDGF; they may also develop from epithelial cells or from bone marrow precursor cells called fibrocytes. So efficient are these myofibroblasts that large wounds may decrease in size by up to 80%.[32]

When the drama ends badly. Several systemic factors may adversely affect the performance of the tissue repair drama. Patients with diabetes mellitus tend to have less granulation tissue formation, less collagen deposition and defects in collagen maturation, leading to slow and ineffective wound maturation.[33] While this predisposition has been attributed to the microangiopathy inherent in diabetes, recent research indicates that other factors, such as collagen glycosylation and pericapillary albumin deposition, may play a role.[22,34] Poor nutrition also retards the healing process. Vitamin C, in particular, is necessary for collagen synthesis. Deprivation of this vitamin results in failure of activation of proline and lysine hydroxylases and formation of unhydroxylated procollagen peptides, resulting in an unstable,

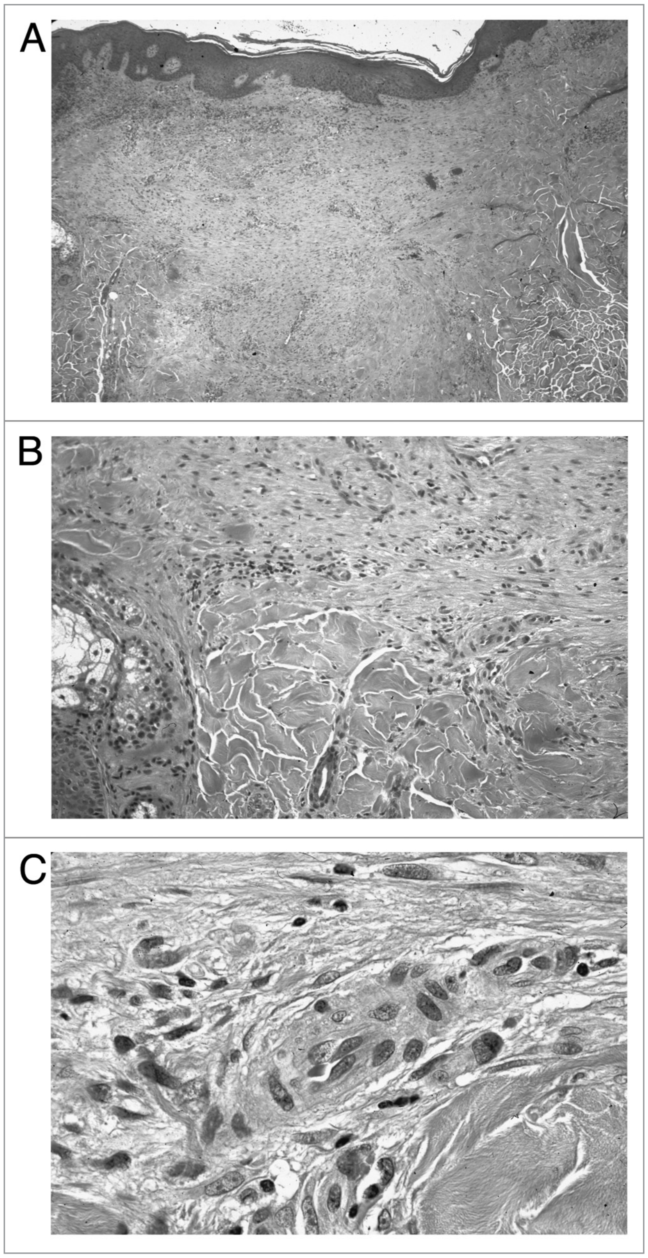

Figure 5. Wide excision of skin. (A) Previous punch biopsy site (center) in late stages of repair. (B) Dense, acellular, normal dermal collagen bundles (bottom) abutting new, still-cellular fibrous tissue of the repair process (top). (C) Blood vessel in process of involution.

poorly cross-linked collagen helix. Conditions in which blood flow is compromised—such as cardiac insufficiency or arteriosclerosis—delay the delivery of necessary cells and factors to the scene of the wound. Certain drugs may also slow wound healing; glucocorticoids, for example, inhibit inflammation and collagen formation.

Local factors can also greatly affect the quality of wound healing. The size of the wound is important (small injuries heal faster than large ones) as is the location (wounds in richly-vascularized areas of the body, such as the face, heal relatively quickly). The presence of foreign bodies may prolong healing, as may motion or pressure at the wound site. However, the single most important cause of delayed healing of wounds is infection.[1] If any beta-hemolytic Streptococcus organisms are present or if there are over 10^5 organisms of any bacterial species per gram of tissue, the wound will not heal.[35] More common in second-intention healing, infection prolongs the inflammatory phase of wound healing, impeding epithelialization, collagen deposition and wound contraction. Bacterial endotoxins stimulate collagenase secretion, leading to degradation of not only the forming scar, but of surrounding normal tissue.[22]

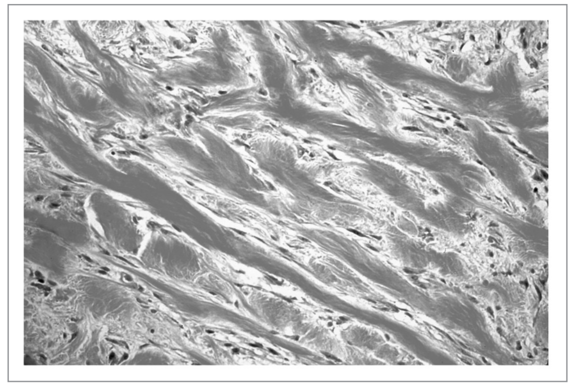

Figure 6. Keloid showing dense, haphazardly-arranged collagen bundles.

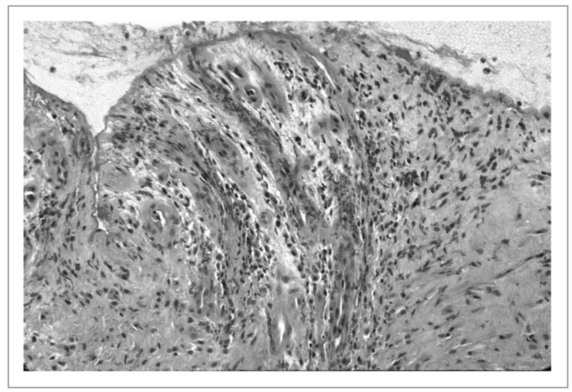

Figure 7. Proud flesh with exuberant granulation tissue.

On the other hand, sometimes acts in the drama are played so well, so exuberantly, that the end result is an abnormally-healed wound. Occasionally, the balance of collagen formation and degradation tips in favor of formation, leading to hypertrophic scars or keloids. Hypertrophic scars are raised scars that remain confined to the region of the wound. They generally occur within 4 weeks of injury (often a severe traumatic or thermal injury to the dermis) and may regress over time.[22,36] An aberrant autocrine loop in which myofibroblasts produce excessive TGF-β and, hence, collagen, appears to contribute to their formation.[36] Keloids are scars that have overgrown the boundaries of the initial incision, presenting as nodules or masses of fibrous tissue (**Fig. 6**). While keloids generally appear within one year of the inciting injury, some may begin growing years later.[22] For unknown reasons, keloids occur much more frequently in patients with darkly-pigmented skin; certain people seem to have a predisposition towards their formation.

Formation of granulation tissue may likewise occur in an excessive fashion. In a lesion known as proud flesh, the process of wound healing is interrupted by masses of granulation tissue protruding above the skin surface, preventing re-epithelialization and appropriate scar formation (**Fig.** 7).

Conclusion

Wound healing is a carefully-scripted drama performed countless times throughout a human lifespan. Though occasionally the drama does not go as planned, in most cases damage is contained, dead cells and tissue are removed and a scar restores integrity to the injured tissue—a triumphant performance indeed.

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